

Application Serial No. 09/981,289  
Filing Date: October 15, 2001

### AMENDMENTS TO THE CLAIMS

The following listing of claims will replace all prior versions, and listings, of claims in this application.

#### Listing of Claims

Claims 1-49 (canceled)

50. (Currently Amended) A variant TNF- $\alpha$  protein comprising an amino acid sequence comprising an amino acid substitution as compared to amino acids 1-157 of SEQ ID NO:2, said substitution at a position selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140, wherein said variant protein interacts with a human TNF- $\alpha$  to form mixed trimers that have a reduced capacity to effect TNF- $\alpha$  receptor signaling in a caspase activity assay.

51. (Previously Presented) A variant protein according to claim 50 wherein said variant protein comprises 2 substitutions at positions selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140.

52. (Previously Presented) A variant protein according to claim 50 wherein said variant protein comprises 3 substitutions at positions selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140.

53. (Previously Presented) A variant protein according to claim 50 wherein said variant protein comprises 4 substitutions at positions selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140.

54. (Previously Presented) A variant protein according to claim 50 wherein said variant protein comprises 5 substitutions at positions selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140.

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55. (Previously Presented) A variant protein according to claim 1 comprising a covalent modification.

56. (Previously Presented) A variant protein according to claim 55 wherein said covalent modification comprises a polyethylene glycol molecule.

57. (Previously Presented) A variant protein according to claim 50 further comprises a substitution at position 145.

58. (Currently Amended) A TNF- $\alpha$  variant protein comprising an amino acid sequence comprising an amino acid substitution as compared to amino acids 1-157 of SEQ ID NO:2, said substitution selected from the group consisting of Q21R, Q21K, N30E, R31V, R31L, N30D, R31I, R31D, R32D, R32E, R32S, R32H, R32T, A35T, A35S, G66Q, G66K, G66N, G66R, G66E, A111R, A111E, A111K, A111D, K112D, K112E, Y115Q, Y115K, Y115E, Y115N, Y115R, Y115F, Y115H, Y115M, Y115L, Y115I, Y115D, Y115T, Y115S, Y115V, D140R, D140K, D140Q, D140E, F144Q, F144H, F144N, E146N, E146K, E146D, E146K, E146Q, E146H, E146E, E146T and E146S, wherein said variant protein interacts with a naturally occurring human TNF- $\alpha$  to form mixed trimers that have a reduced capacity to effect TNF- $\alpha$  receptor signaling in a caspase activity assay.

59. (Currently Amended) A method of forming mixed TNF- $\alpha$  trimers comprising combining:

a) a first variant TNF- $\alpha$  trimer comprising a variant TNF- $\alpha$  protein comprising an amino acid sequence having an amino acid substitution as compared to amino acids 1-157 of SEQ ID NO:2, said substitution at a position selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140; and

b) a human TNF- $\alpha$  trimer;

under conditions whereby mixed trimers are formed that have a reduced capacity to effect TNF- $\alpha$  receptor signaling in a caspase activity assay.

60. (Previously Presented) A method according to claim 59 wherein said first variant trimer has two variant monomers.

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61. (Previously Presented) A method according to claim 60 wherein said first variant trimer has three variant monomers.

62. (Previously Presented) A method according to claim 59 wherein said variant protein comprises a covalent modification.

63. (Previously Presented) A method according to claim 62 wherein said covalent modification comprises a polyethylene glycol molecule.

64. (Currently Amended) A TNF- $\alpha$  mixed trimer comprising a variant TNF- $\alpha$  protein comprising an amino acid sequence having an amino acid substitution as compared to amino acids 1-157 of SEQ ID NO:2, said substitution at a position selected from the group consisting of positions 21, 30, 31, 32, 35, 66, 111, 112, 115 and 140, wherein said mixed trimers have a reduced capacity to effect TNF- $\alpha$  receptor signaling in a caspase activity assay.

65. (Previously Presented) A mixed trimer according to claim 64 wherein one of the monomers in said trimer is a human TNF- $\alpha$  monomer.

66. (Previously Presented) A mixed trimer according to claim 64 wherein two of the monomers in said trimer are independently selected variant TNF- $\alpha$  proteins.

67. (Previously Presented) A mixed trimer according to claim 66 wherein said variant TNF- $\alpha$  proteins are the same.

68. (Previously Presented) A mixed trimer according to claim 66 wherein said variant TNF- $\alpha$  proteins are different.

69. (Previously Presented) A mixed trimer according to claim 64 where all of the monomers in said trimer are variant TNF- $\alpha$  proteins.

70. (Currently Amended) A TNF- $\alpha$  mixed trimer comprising a variant TNF- $\alpha$  monomer comprising an amino acid sequence having an amino acid substitution as compared to amino acids 1-157 of SEQ ID NO:2, wherein said substitution is selected from the group consisting of K112D, Y115T, Y115I, D143K and D143R.

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71. (Currently Amended) A mixed trimer according to 70 further comprising a second variant monomer having an amino acid sequence with an amino acid substitution as compared to amino acids 1-157 of SEQ ID NO:2.

72. (Currently Amended) A TNF- $\alpha$  mixed trimer comprising a variant TNF- $\alpha$  protein comprising an amino acid substitution as compared to amino acids 1-157 of SEQ ID NO:2, wherein said substitution is selected from the group consisting of Q21R, Q21K, N30E, R31V, R31L, N30D, R31I, R31D, R32D, R32E, R32S, R32H, R32T, A35T, A35S, G66Q, G66K, G66N, G66R, G66E, A111R, A111E, A111K, A111D, K112D, K112E, Y115Q, Y115K, Y115E, Y115N, Y115R, Y115F, Y115H, Y115M, Y115L, Y115I, Y115D, Y115T, Y115S, Y115V, D140R, D140K, D140Q, D140E, F144Q, F144H, F144N, E146N, E146K, E146D, E146K, E146Q, E146H, E146E, E146T and E146S.